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EFFECTS OF NITROGEN ON GROWTH AND ASH CONSTITUENTS OF *ANANAS COMOSUS* (L.) MERR.[1]

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(WITH EIGHT FIGURES)

General

The preferred nitrogenous fertilizer in commercial plantations of pineapples is ammonium sulfate, according to JOHNSON (**16**). When sulfate of ammonia is used, the ammonium ion is oxidized to nitrate by soil microbiological activity, with an associated increase in soil acidity which renders iron more soluble. At the same time, both ammonium and nitrate ions are generally simultaneously available to the pineapple roots. Sodium nitrate is generally inferior and usually produces an atypical light-green color in leaves and unripe fruit of the pineapple. When sodium nitrate is used under dry conditions, however, there may be accumulation of sodium bicarbonate in the soil as reported by KELLEY and THOMAS (**17**) caused by a greater rate of uptake of nitrate than of sodium by roots and by soil microflora, a phenomenon likely to cause precipitation of iron in the soil and associated plant chlorosis. Efficient nitrogen fertilization of pineapple plants depends greatly on an understanding of the interrelationships of nitrate and ammonium ions with (a) the inorganic nutrient complex of the soil, that is, the relative concentrations of nutrient elements other than NO_3^- or NH_4^+; (b) the activities of the microflora in the soil; and (c) shifting of pH values, and changes of temperature and moisture affecting the concentrations of these ions in the soil. It is also important to know the nitrogen requirements of plants at different growth stages on the basis of nitrogen inventories within the soil and plant tissues.

This paper reports plant weights attained by one-year-old *Ananas comosus* grown in solution cultures with 140.0 or 2.8 mg. of nitrogen in nitrate or ammonium form and the content of ash, water, relative electrical resistance, potassium, calcium, magnesium, phosphorus and iron in the tissue.

[1] Published with the approval of the Director as Technical Paper no. 164 of the Pineapple Research Institute, University of Hawaii.

Cultural, chemical and statistical methods

Crowns, i.e., vegetative organs produced at the apical end of fruits, weighing approximately 100 gm. after sun curing and stripping of dried vestigial leaves, were suspended with bases in tap water for two weeks until roots developed. Plants with approximately 25 roots with an average length of 10 cm. were then selected in groups of 16 per culture and transferred to five-gallon porcelain crocks containing the nutrient solutions reported in table I. These solutions, constantly aerated and their acidity adjusted within a range of pH 4.5 to 6.5, were renewed at two-week intervals. Fourteen plants were removed from each nutrient solution after one year's growth

TABLE I

COMPOSITION OF VARIOUS NUTRIENT SOLUTIONS

Salts	Salt per 100 liters (Grams)				Elements	Elements per milliliters (Micrograms)			
	Series					Series			
	Nitrate		Ammonium			Nitrate		Ammonium	
	High-N*	Low-N*	High-N	Low-N		High-N	Low-N	High-N	Low-N
	gm.	*gm.*	*gm.*	*gm.*		γ	γ	γ	γ
$Ca(NO_3)_2 \cdot 4H_2O$	118.08	2.36	0.00	0.00	N	140.0	2.8	140.0	2.8
$(NH_4)_2SO_4$	0.00	0.00	66.07	1.32	Ca	200.0	20.0	200.0	20.0
KH_2PO_4	6.81	6.81	6.81	6.81	K	48.8	48.8	48.8	48.8
K_2SO_4	6.53	6.53	6.53	6.53	P	15.5	15.5	15.5	15.5
$CaCl_2$	0.00	10.10	56.00	11.20	S	18.1	18.1	178.1	21.3
$MgSO_4 \cdot 7H_2O$	24.65	24.65	24.65	24.65	Mg	12.2	12.2	12.1	12.2
$FeSO_4 \cdot 7H_2O$	1.39	1.39	1.39	1.39	Fe	2.7	2.7	2.7	2.7
$ZnSO_4 \cdot 7H_2O$	0.29	0.44	0.44	0.44	Zn	0.6	0.6	0.6	0.6
H_3BO_3	0.28	0.28	0.28	0.28	B	0.5	0.5	0.5	0.5
$H_2MoO_4 \cdot H_2O$	0.02	0.02	0.02	0.02	Mo	0.1	0.1	0.1	0.1
$MnSO_4 \cdot 6H_2O$	0.26	0.26	0.26	0.26	Mn	0.5	0.5	0.5	0.5
					Cl	0.0	65.0	360.0	72.0

* High-N = 140.0 mg. of nitrogen per liter.
Low-N = 2.8 mg. of nitrogen per liter.

and segregated into roots, leaves and stem. The leaves were further segregated into different age groups, and these and the stems were sectioned on the basis of differences in physiological functions in accordance with a technique reported elsewhere (**30**). Chemical methods for the analysis of the various constituents of the tissues have also appeared in former publications (**27, 28, 29, 31, 32**).

Calculation of the significance of the difference of the means of plant weights between different cultures was made by FISHER's method (**10**).

$$t = \frac{\bar{x} - \bar{x}^1}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 2}}$$

Observations

PLANT GROWTH

Table II shows that leaf and stem weights of the high-N cultures were

greater than of the low-N cultures, but root weights were relatively greater in the low-N than high-N cultures.

Ratios of weights of leaf, stem or root to plants for the various cultures are reported in table III and indicate that root weights in the low-N cultures gained at the expense of leaf and stem weights whereas in the high-N cultures the opposite condition obtained.

TABLE II

MEAN WEIGHTS OF TOTAL PLANTS, STEMS, LEAVES AND ROOTS OF ONE-YEAR-OLD PLANTS WITH CALCULATED "t" VALUES

SERIES OF	ITEMS COMPARED	N	MEAN WEIGHTS		CALCULATED "t" VALUES†
			HIGH-N*	LOW-N*	
			gm.	*gm.*	
Nitrate-N	Total plants	14	3650	2375	11.64
Ammonium-N	" "	14	3070	2605	2.60
Nitrate-N	Stems	14	376	147	6.00
Ammonium-N	"	14	254	167	3.37
Nitrate-N	Leaves	14	2952	1755	26.30
Ammonium-N	"	14	2660	2035	6.43
Nitrate-N	Roots	14	322	473	6.00
Ammonium-N	"	14	156	403	13.72

* High-N = 140.0 mg. of nitrogen per liter.
Low-N = 2.8 mg. of nitrogen per liter.
† All "t" values indicated highly significant differences between means (p less than 0.01) with the exception of value of 2.60, where significance level was slightly greater than p = 0.02.

The ratios of stem or root to plant weights indicate that in the high-N cultures, stems and roots but not leaves, comprised a greater proportion of the total plant in the nitrate than in the ammonium series.

WATER

The water content of tissues, reported in figure 1, was generally higher in the high-N than in the low-N cultures in both series in the basal (no. 1) and transitional (no. 2) sections of the mature (C) and active (D) leaves and in the roots. Also, in the ammonium series it was higher in similar sections of the young (E) leaves and in the stem. The water content of all other sections, except the basal stem sections of the high-N cultures in the nitrate series, was higher in the low-N than in the high-N cultures in both series.

TABLE III

RATIOS OF WEIGHTS OF LEAVES, STEMS OR ROOTS TO PLANTS IN THE VARIOUS CULTURES

ITEMS OF COMPARISON	NITRATE SERIES		AMMONIUM SERIES	
	HIGH-N	LOW-N	HIGH-N	LOW-N
Leaves to plants	0.810	0.739	0.866	0.782
Stems to plants	0.102	0.062	0.083	0.064
Roots to plants	0.088	0.199	0.051	0.154

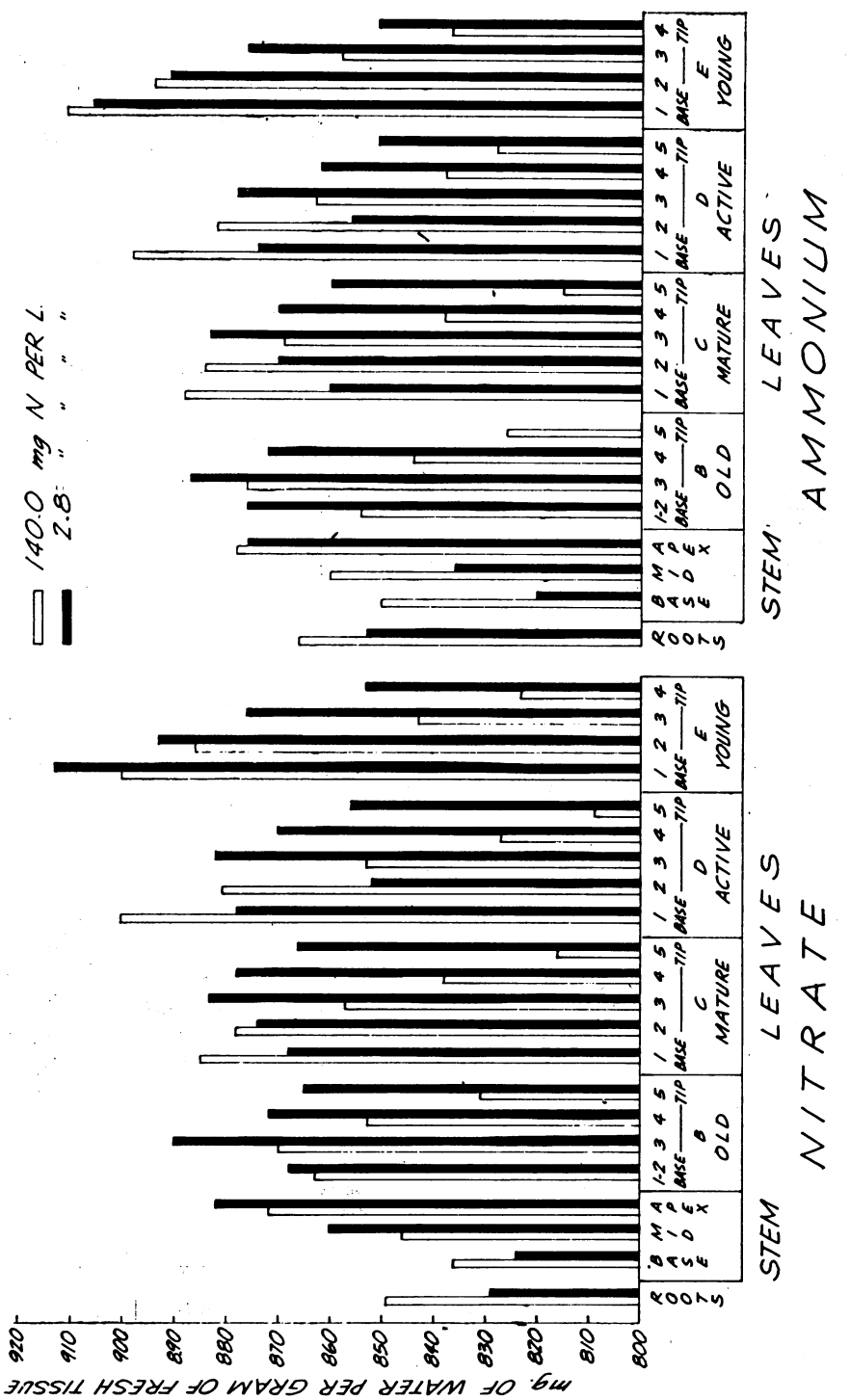

FIG. 1. Water in plant sections.

Differences in the moisture content of comparable sections between high-N and low-N cultures had resulted either from differences in the amounts of meristematic to differentiated tissues in the basal (no. 1) and transitional (no. 2) sections of the mature (C), active (D) and young (E) leaves, or from differences in the amounts of stored organic or inorganic solutes in the chlorophyllose (no. 3, 4 and 5) sections of the leaves. Similar differences in the moisture content of the stem may also be accounted for by the differences in the starch content.

Ash

Ash values, reported as mg. per gram of fresh tissue, in figure 2, were higher in the low-N than high-N cultures in all sections except the roots and the basal and medial stem sections of the nitrate series. However, the higher ash content of the low-N than high-N cultures in the nitrate series may be attributed to concentration effects which resulted from the lower plant weights in the former than in the latter cultures. Differences in the ash content of comparable sections between high-N and low-N cultures were generally greater in the ammonium than in the nitrate series. In the ammonium series the differences had possibly resulted from the antagonistic effects of high concentrations of NH_4 ions in the high-N cultures on the absorption of other cations by roots, especially Ca ions, which effects were lacking in the high-N cultures of the nitrate series.

Total ash values per plant, reported in table IV, show that the high-N cultures in the nitrate series contained 40 per cent. more ash than the low-N cultures. However, in the ammonium series the ash content of the low-N cultures was 21.5 per cent. higher than that of the high-N cultures. The data further emphasize the fact that the low ash content of the high-N cultures in the ammonium series had resulted from a reduction of the rate of intake by roots of potassium, calcium and magnesium through the antagonistic effects of ammonium ions. In the nitrate series, although the ash content reported as mg. per gram of fresh tissue in figure 2 was higher in the low-N than high-N cultures, total ash values per plant, in table IV, were greater for the high-N than low-N cultures, indicating that the lower plant weights of the low-N cultures rather than a greater absorption of ash constituents raised the concentrations of the latter to higher levels in the low-N than high-N cultures.

Electrical resistance

Values of the relative electrical resistance (ohms) of the extracted sap, reported in figure 3, were indirectly proportional to the amounts of ash constituents. Factors directly affecting relative electrical resistance values are the concentration and degree of ionization of various inorganic and organic solutes in the sap, the latter not reported here because of their destruction during ashing. Electrical resistance values, being inversely proportional to the ionic content of the sap, were greater in the high-N than in the low-N cultures in conformity with the salt content in the tissues of these cultures, as reported in figure 2. For example, comparison of the data in figures 2

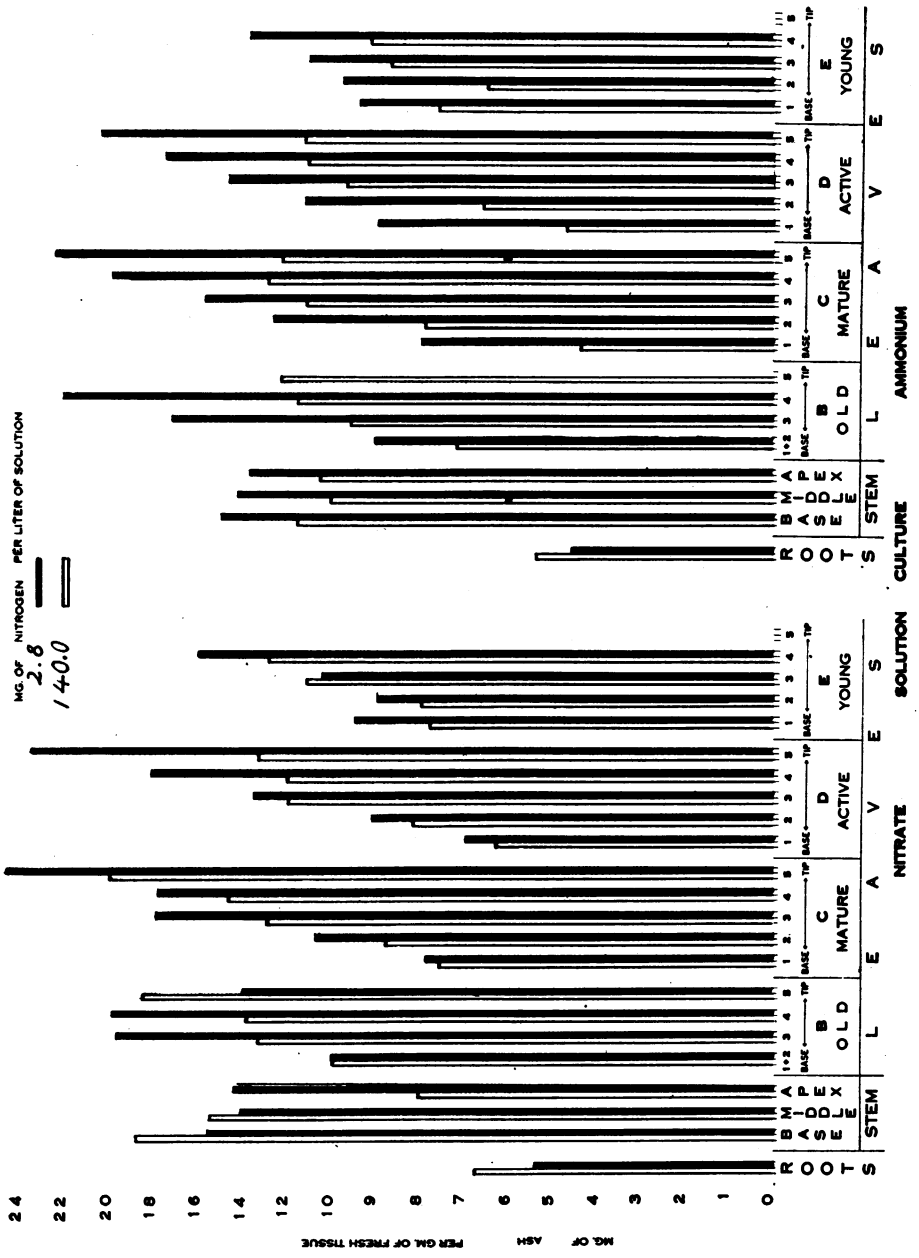

FIG. 2. Ash in plant sections.

TABLE IV

AMOUNTS OF VARIOUS ASH CONSTITUENTS PER TOTAL PLANT, OR ORGANS OF *Ananas comosus*, GROWN IN HIGH-NITROGEN OR LOW-NITROGEN CULTURES

ITEMS		NITRATE SERIES								AMMONIUM SERIES							
ELEMENTS GM./PLANT	UNITS OF MEASURE	HIGH-N*				LOW-N*				HIGH-N				LOW-N			
		PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Ash	mg.	44,331	37,116	5,025	2,190	31,643	26,872	2,186	2,585	29,224	25,728	2,650	846	35,492	31,257	2,400	1,835
K	"	18,787	17,032	1,069	686	17,588	15,548	744	1,296	13,282	11,921	1,063	298	25,522	22,527	1,375	1,620
Ca	"	6,680	4,686	1,736	258	1,115	654	351	110	728	485	215	28	1,090	727	309	55
Mg	"	1,039	886	92	61	1,056	791	103	162	967	720	196	51	1,424	1,171	155	99
P	"	766	624	99	43	423	294	30	99	1,004	927	60	17	532	460	30	42
Fe	γ	24,142	2,074	268	21,800	22,325	1,954	171	20,200	6,185	3,547	259	2,379	16,587	1,654	143	14,790

* High-N = 140.0 mg. of nitrogen per liter.
Low-N = 2.8 mg. of nitrogen per liter.

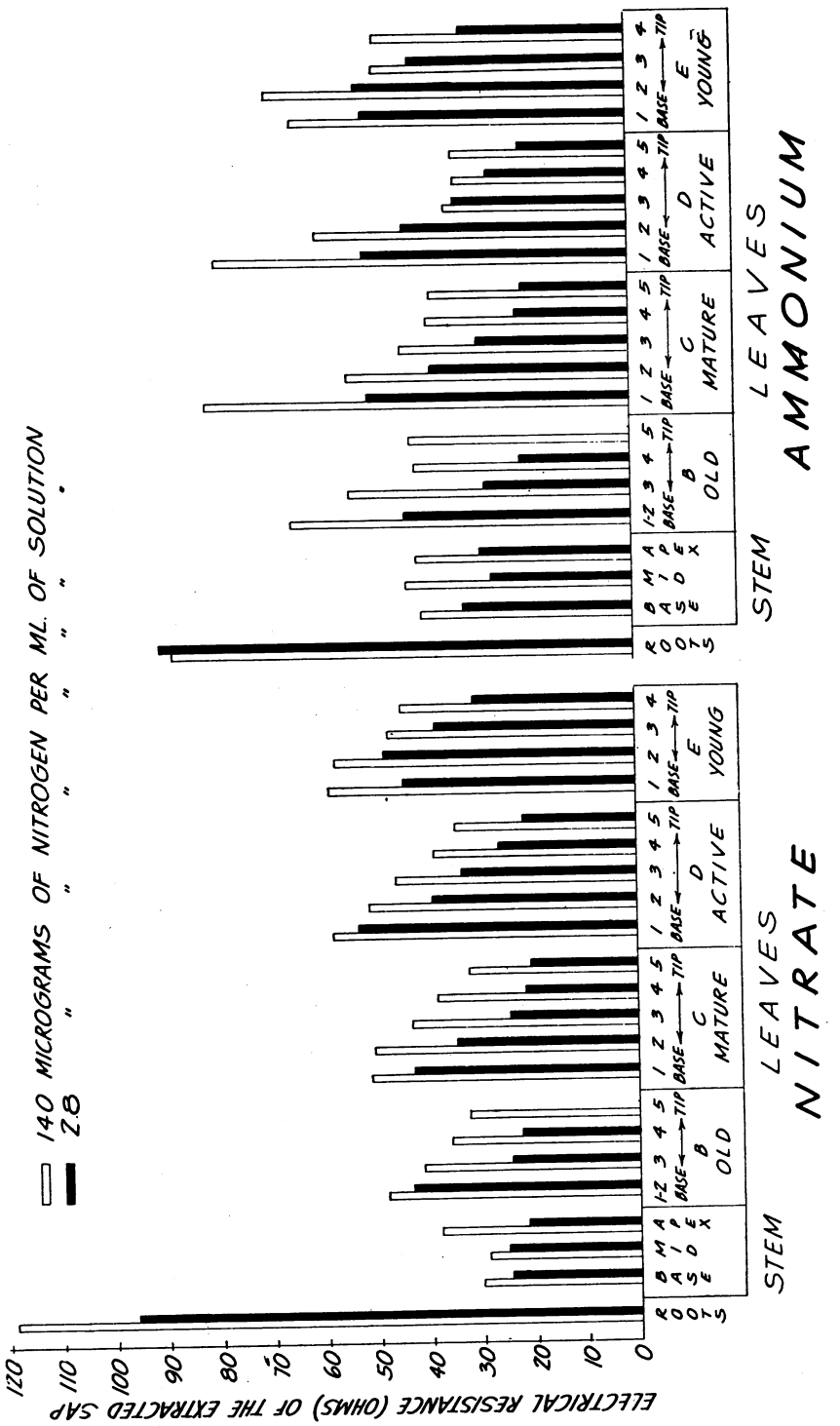

FIG. 3. Relative electrical resistance (ohms) of plant sections.

and 3 reveals that the tissues of the terminal leaf sections (no. 5), being chronologically more advanced than of other sections in the same leaves, contained more salt and had lower values for electrical resistance than the less advanced tissues in other sections. However, this rule did not apply to the meristematic tissues of the basal sections (no. 1) of the young (E) leaves which, because of a greater growth rate presumably possessed a higher degree of metabolic activity and required possibly greater salt concentrations than the chronologically more advanced tissues of adjacent sections with a reduced rate of growth.

Potassium

Potassium, reported in figure 4 as mg. per gram of fresh tissue, was higher for the low-N than high-N cultures in both nitrogen series except for the terminal (no. 4) sections of the old (B) leaves in the nitrate series. Comparison of histogram heights in comparable cultures between the nitrate and ammonium series shows that potassium values for the high-N cultures were higher in the nitrate than ammonium series, whereas for the low-N cultures they were reversed. Total potassium values per plant, reported in table IV, were greater for the high-N than low-N cultures in the nitrate series, but in the ammonium series similar values were greater for the low-N than high-N cultures.

The data reveal that potassium absorption by roots from nutrient solutions was enhanced by the high content of NO_3 ions in the high-N cultures of the nitrate series, but in the ammonium series it was greatly retarded by the high concentration of NH_4 ions.

Calcium

Calcium values, reported in figure 5 as mg. per gram of fresh tissue, were greater for the high-N cultures in the nitrate series and the low-N cultures in the ammonium series than for the opponent cultures in either series. The effects of nitrate-ions enhancing calcium absorption from nutrient solutions by roots and of ammonium-ions retarding calcium absorption are clear cut.

Comparison of calcium values in the stem with those in the leaves shows that in the former organ they were many times greater than in the latter, possibly suggesting a higher rate of calcium absorption by the roots and transportation to stem than of translocation from stem to leaves.

Total calcium values per plant, reported in table IV, were higher for the high-N cultures in the nitrate series and for the low-N cultures in the ammonium series than for the low-N cultures in nitrate and high-N cultures in the ammonium series. The data on calcium, calculated either as mg. per gram of tissue or as total calcium per plant, indicated that NO_3 ions in the nitrate series increased and NH_4 ions in the ammonium series decreased the calcium content of tissues. Calcium differences between high-N and low-N cultures in the nitrate series, being greater than plant weight differences between the same cultures, indicate that the influence of high concentrations of NO_3 ions in the high-N cultures enhancing calcium absorption was greater than the

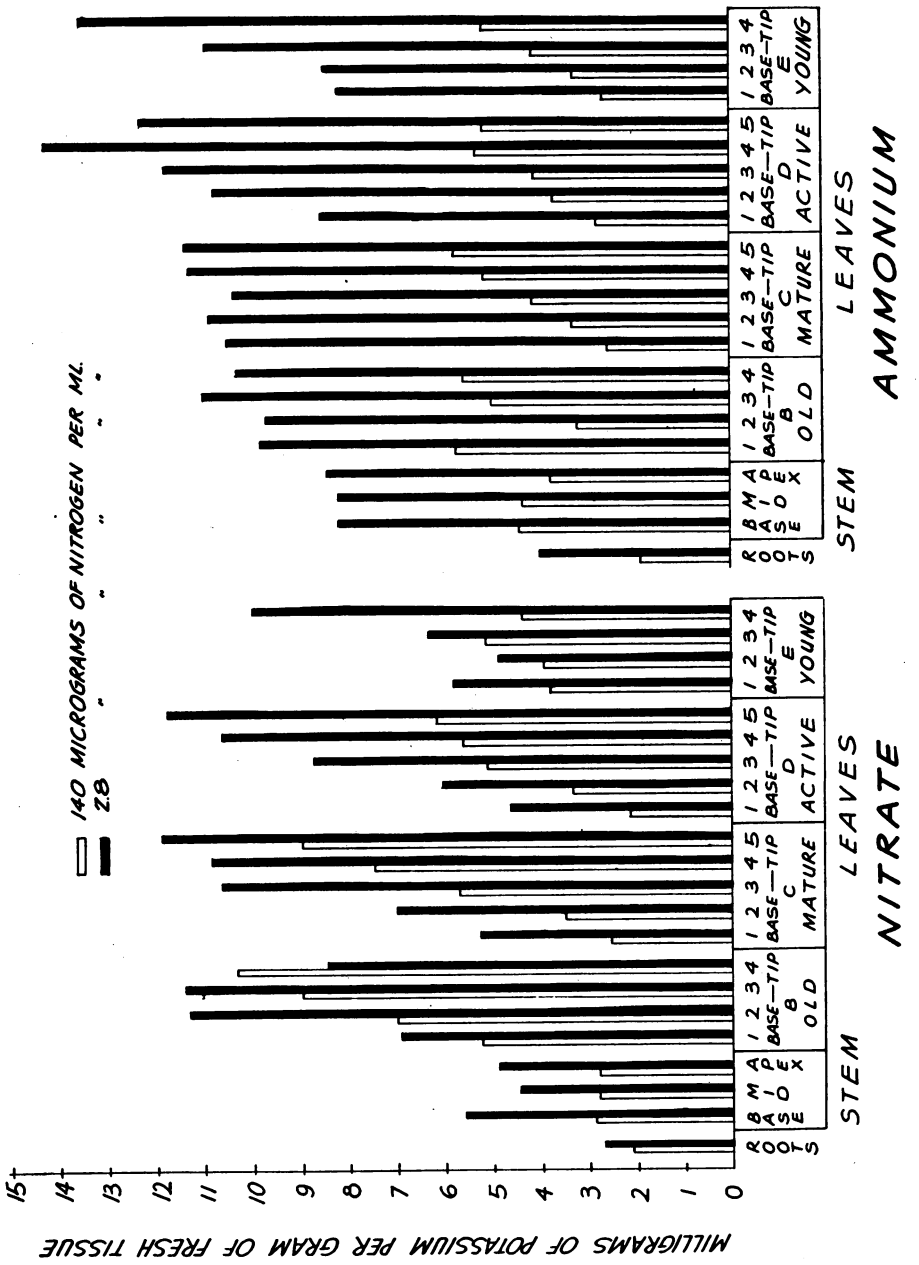

FIG. 4. Potassium in plant sections.

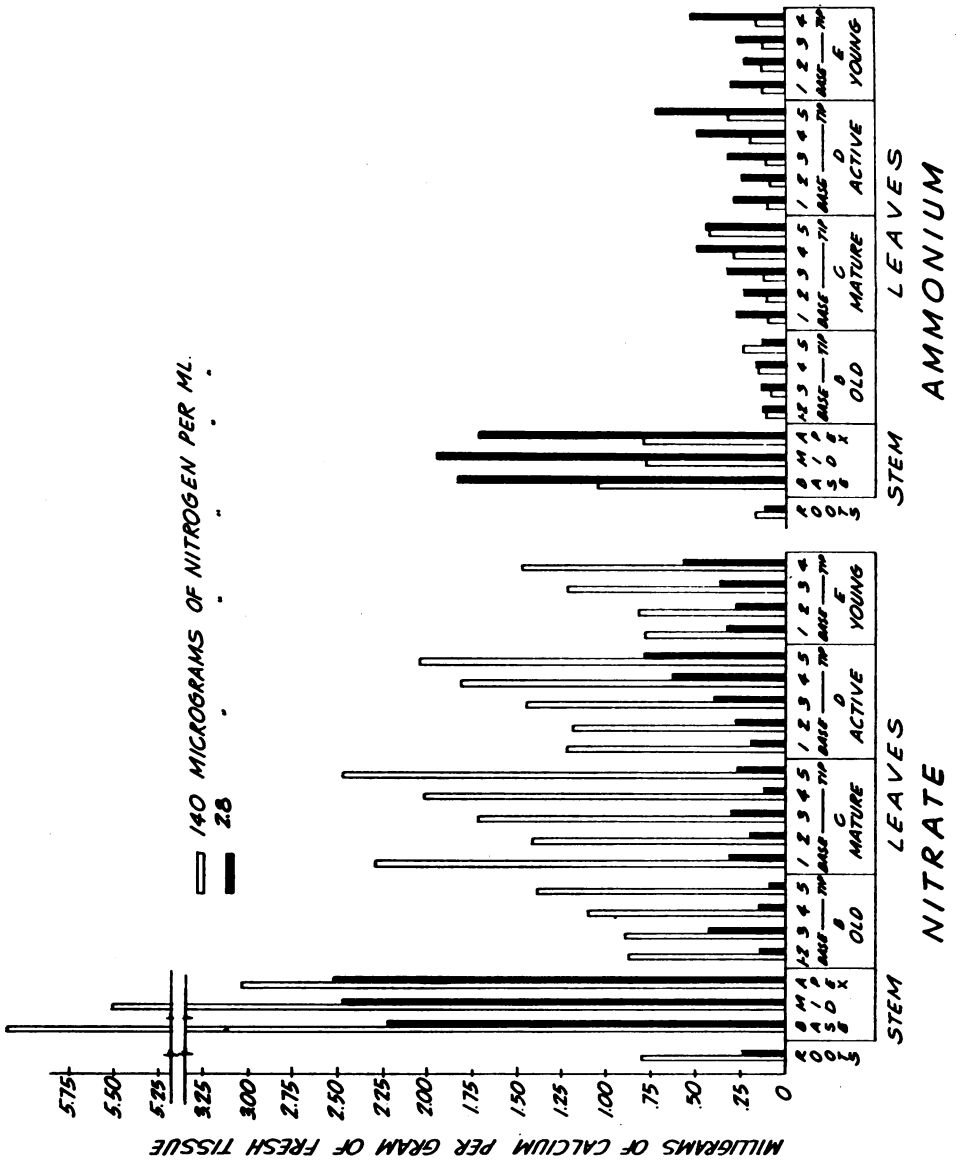

FIG. 5. Calcium in plant sections.

tendency for accumulation of calcium in the low-N cultures of the same series because of retarded growth and small plant volumes.

Magnesium

Magnesium values, reported in figure 6 as mg. per gram of fresh tissue, were greater for the low-N than for the high-N cultures in both nitrogen series.

Total magnesium values per plant, in table IV, were approximately the same in the high-N and low-N cultures of the nitrate series, but in the ammonium series the low-N cultures contained approximately 47 per cent. more magnesium than the high-N cultures. It is possible that the difference in magnesium between high-N and low-N cultures in the ammonium series had resulted from a decreased rate of intake of Mg ions caused by the antagonistic effects of the high concentration of ammonium ions.

Accumulation of magnesium as percentage of total in the stem of the high-N cultures was 8.85 and 20.30 per cent. for nitrate and ammonium series, respectively, indicating a higher rate of absorption of magnesium and transport to the stem but a retarded rate of translocation from the stem to the leaves in the latter than former series. In the low-N cultures the differences in the magnesium content of stem and leaf tissues between nitrate and ammonium series were not as great as in the high-N cultures.

Phosphorus

Phosphorus values, reported in figure 7 as mg. per gram of fresh tissue, were generally greater in the ammonium than in the nitrate series. Phosphorus differences between high-N and low-N cultures were in favor of the former cultures in both nitrogen series.

Total phosphorus values per plant, in table IV, were higher in the ammonium than nitrate series and were in agreement in this respect with the values in figure 7, suggesting that PO_4 ions were attracted electrostatically by NH_4 ions in the ammonium series and more so by the high-N than low-N cultures. However, comparable electrostatic repelling of PO_4 ions by NO_3 ions could not be observed in the high-N cultures of the nitrate series because these cultures showed 1.8 times as great intake of PO_4 ions as the low-N cultures of the same series. Phosphate concentrations in the nutrient solutions being relatively small, the conditions for the phenomena of antagonism were probably not propitious.

Iron

The data in figure 8 on the iron content of the various cultures are more involved and less suitable to interpretation than those pertaining to other nutrient elements because of the high susceptibility of iron to precipitation on the epidermal cells of roots during adsorption and its low translocation rate from roots to other plant organs. Such precipitation on the epidermal root layer is greatly influenced by changes in the hydrogen-ion concentration at the interfacial layer, resulting from an unequal absorption of anions and cations.

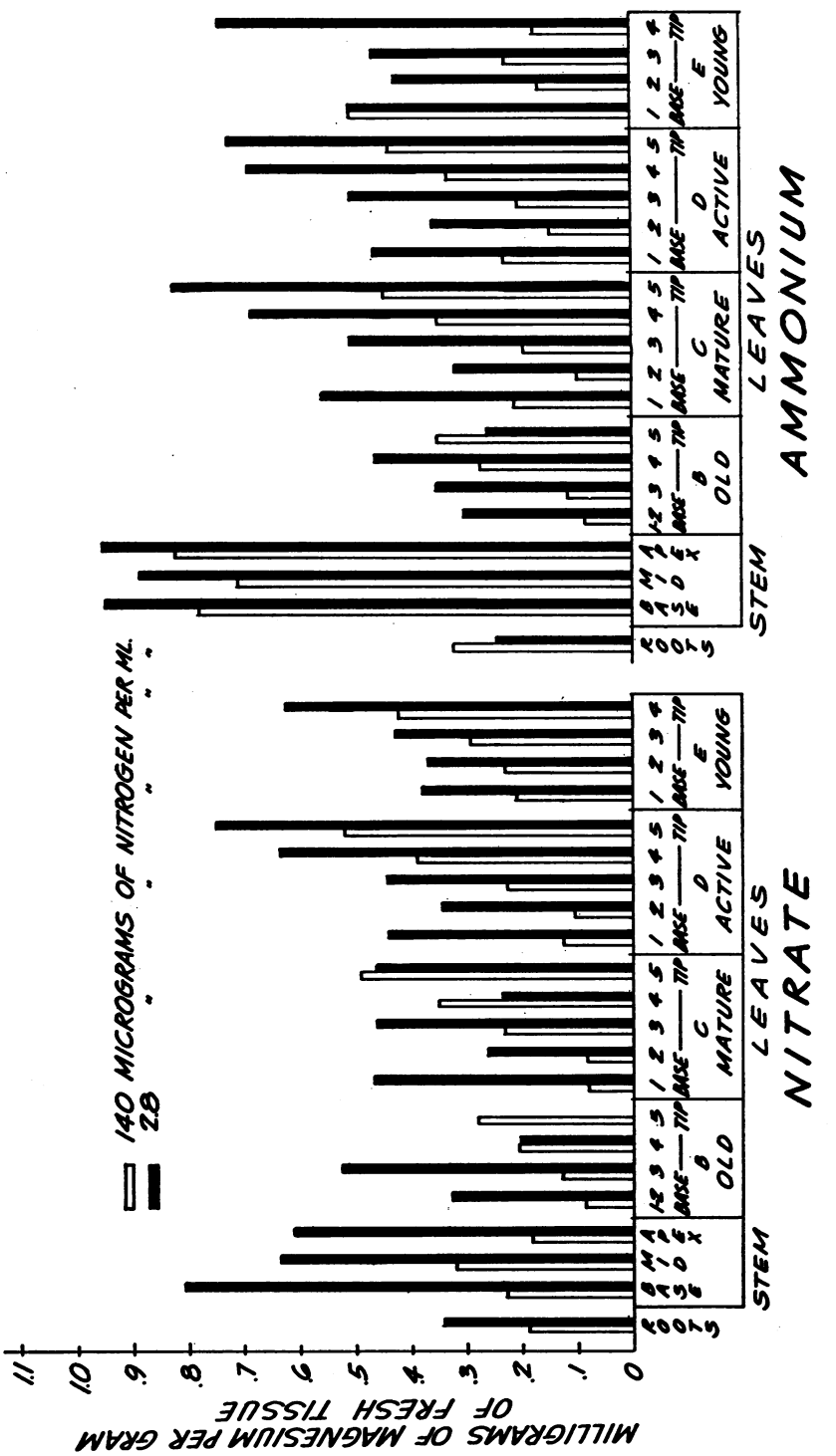

FIG. 6. Magnesium in plant sections.

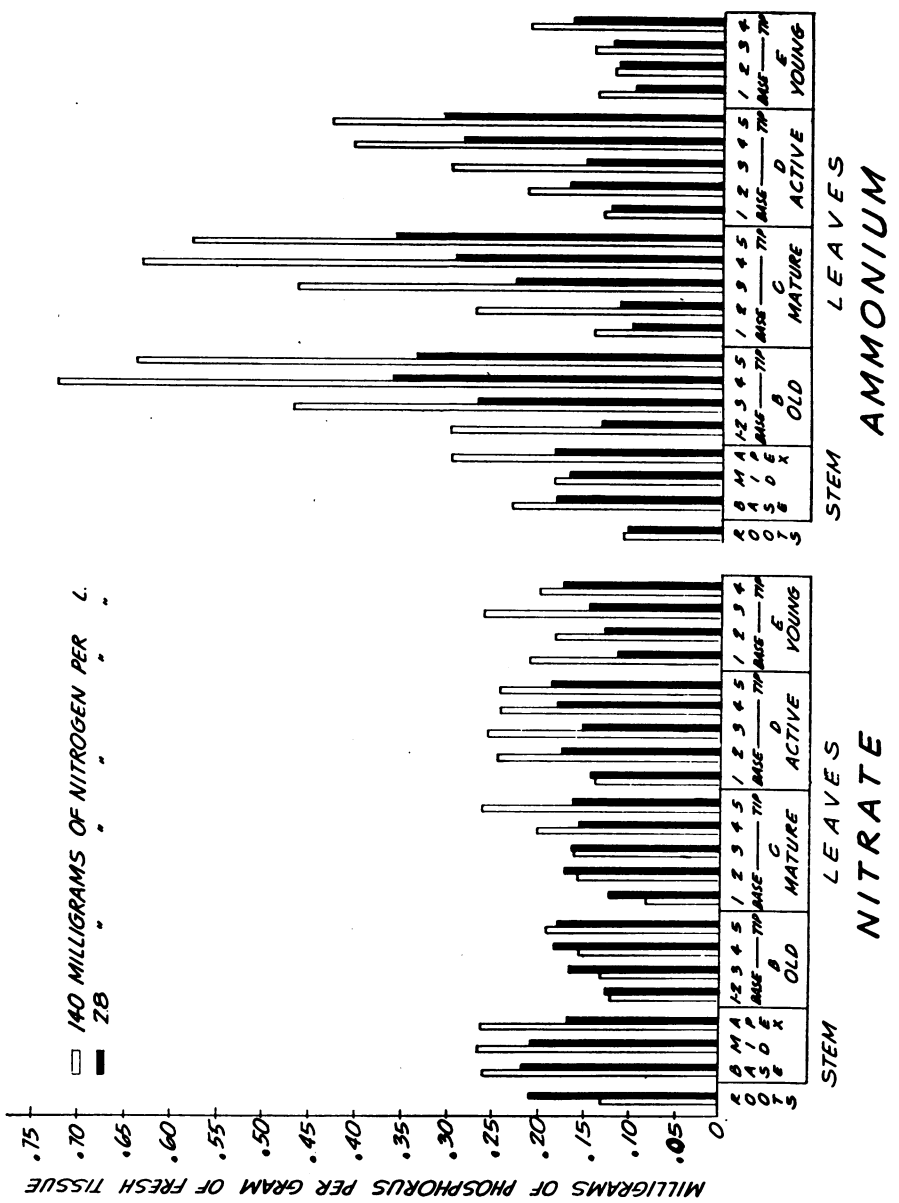

FIG. 7. Phosphorus in plant sections.

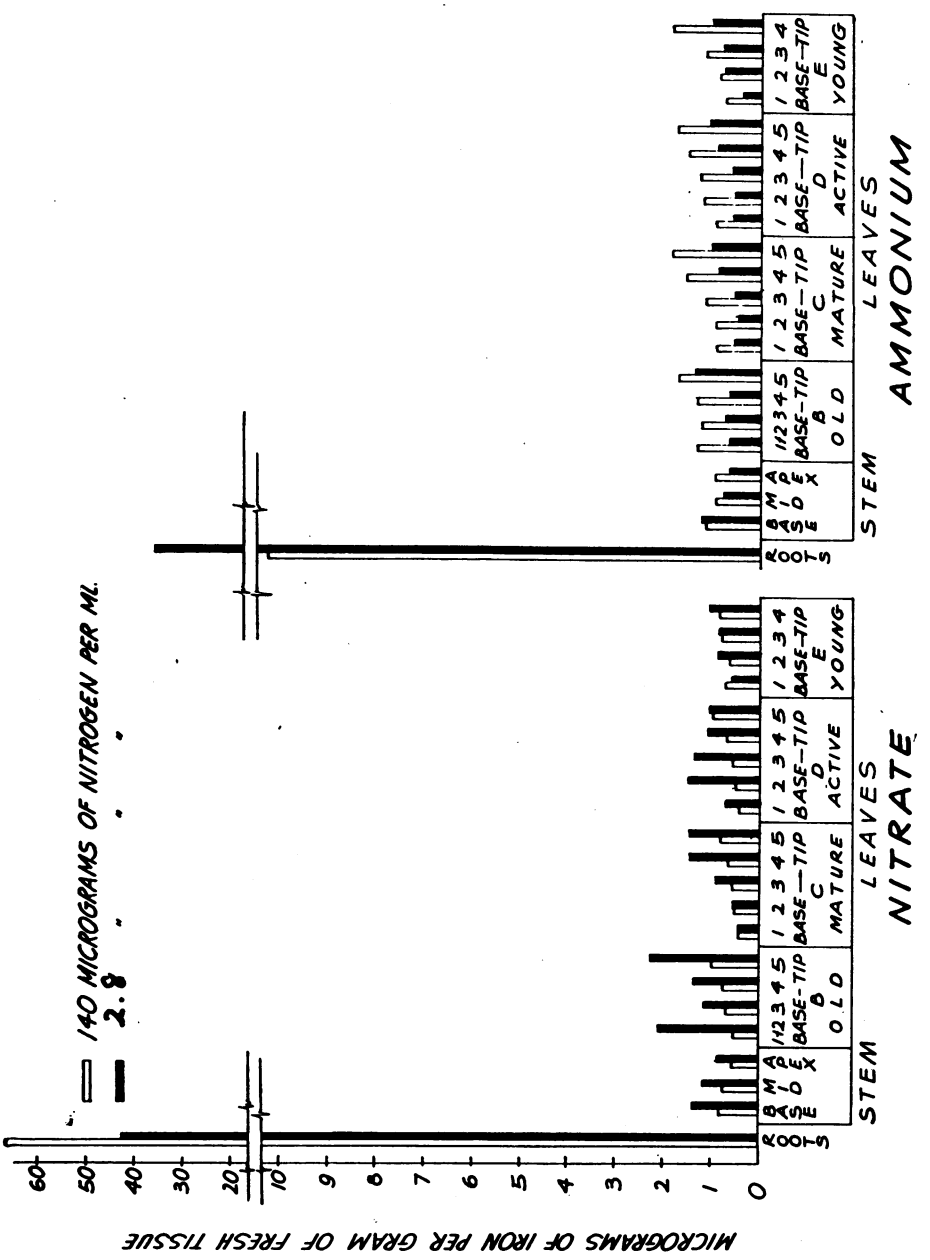

FIG. 8. Iron in plant sections.

Comparison of iron concentrations in the root tissues with the mean iron concentration of all other plant sections shows that in the nitrate series the former were 98.3 and 37.5 times as high as the latter for the high-N and low-N cultures, respectively. In the ammonium series corresponding ratios were 12.1 for the high-N and 47.2 for the low-N cultures. The data reveal that iron precipitation on the epidermal root cells was greatest for the high-N cultures in the nitrate series and smallest for the high-N cultures in the ammonium series. Iron precipitation on the roots of the low-N cultures in both series was intermediate between the high-N cultures of the nitrate series on the one side and ammonium series on the other side. The high iron precipitation in the nitrate series had resulted from the shifting of pH from 4.5 to 6.6 because of greater absorption from the nutrient solution of nitrate than calcium ions; whereas low iron precipitation on the roots of the high-N cultures in the ammonium series had resulted from a countershift in the pH course, e.g., from 6.5 to 4.0 caused by a greater absorption of NH_4^+ than SO_4^- ions and formation of H^+ ions by hydrolysis. Average iron concentrations in the plant, excluding the roots, were greatest in the high-N cultures of the ammonium series and smallest in the high-N cultures of the nitrate series. Comparison of iron concentrations in the roots with the average concentrations in the combined leaf and stem sections shows that in the latter organs iron concentrations were inversely proportional to the amounts of iron precipitated on the roots.

Total iron values per plant, reported in table IV, reveal very little unless considered with respect to its distribution in the various organs. The iron content of the leaves of the high-N cultures in the ammonium series was 1.71 times as great as that of the high-N cultures in the nitrate series. Iron in the leaves of the low-N cultures in the nitrate series was 1.19 times as great as that for comparable cultures in the ammonium series. Differences in the iron content of the stems were smaller between comparable cultures in different series than between different cultures in the same series; for example, the difference in stem iron between the nitrate and ammonium cultures was 3.5 per cent. for the high-N cultures and 19.6 per cent. for the low-N cultures. But, similar differences between high-N and low-N cultures in the same series were 56.7 and 81.2 per cent. in favor of the former cultures for the nitrate and ammonium series, respectively. Differences in the total iron of the roots between the high-N cultures in the nitrate and ammonium series were 816.0 per cent. in favor of the former series. Similar differences between the low-N cultures were 36.5 per cent. in favor of the nitrate series. Differences in total root iron between high-N and low-N cultures in the nitrate series were 7.9 per cent. in favor of the high-N cultures, but similar differences in the ammonium series were 522.0 per cent. in favor of the low-N cultures. The amounts of iron deposited or precipitated in the roots of the high-N cultures (as the result of H-ion changes, mentioned above) were 9.15 times greater for the nitrate than ammonium series, whereas the amounts of iron transported to the leaves were 1.7 times greater for the ammonium than

nitrate series. These results clearly indicate that the greater iron depositions in the roots of the nitrate cultures constituted insoluble iron which was unavailable for translocation to the tissues of the stem and leaves, whereas the smaller iron depositions in the roots of the ammonium series suggest a greater availability for translocation to other organs of the plant. The above results are in agreement with other findings on the effects of NO_3 and NH_4 ions in connection with other studies reported previously (**32, 33**).

Discussion

Synoptic review of the results obtained shows that a fifty-fold increase in the external concentration of nitrogen, i.e., from 2.8 to 140.0 mg. per liter, increased the total nitrogen uptake by plants 4.85 and 5.35 times in the nitrate and ammonium series, respectively. Plant weights were increased 1.537 and 1.180 times in the nitrate and ammonium cultures, respectively. Comparative plant weight increases between the high-N and low-N cultures were lower for the ammonium than nitrate series which should be attributed to the growth inhibiting properties of the chloride content of the former series. Calcium uptake by plants from the nutrient solution was 6.00 times as great for the high-N as low-N cultures in the nitrate series and 0.67 times as great for the high-N as low-N cultures in the ammonium series. Potassium absorption was approximately the same for the high-N and low-N cultures in the nitrate series and magnesium showed the same relationship. In the ammonium series, however, the high-N cultures absorbed 0.52 times as much potassium and 0.68 times as much magnesium as the low-N cultures. Phosphate uptake was 1.81 and 1.88 times greater by the high-N than low-N cultures of the nitrate and ammonium series, respectively. Iron uptake, by the high-N cultures, as determined by total iron in leaves and stem being greater 1.11 and 2.11 times for the nitrate and ammonium series, respectively, was influenced more by changes in the H-ion concentration resulting from the uptake by roots of anions and cations at unequal rates than by the direct effects of different concentrations of nitrate or ammonium ions.

The results as stated above appear to be in satisfactory agreement with results obtained by various investigators with other plants. EATON and RIGLER (**9**) observed in the cotton plant that an increase in nitrate from a low level resulted in an increase in vegetative growth, in an increased number of bolls, and in higher concentrations of nitrates and total nitrogen in the plant tissues. WADLEIGH (**35**) working also with cotton found that increasing the nitrate content of the substratum from 8 to 25, 75, and 225 p.p.m. increased plant nitrate nitrogen from 0.08 to 0.225, 0.80, and 1.38 gm. and yield of seed from 27.0 to 58.1, 119.8, and 143.0 gm. per plant, respectively. Somewhat similar results were obtained by BENSEND (**6**) with different amounts of nitrogen on Jack pine, *Pinus banksiana* Lamb., seedlings which gained sixteenfold in weight as the amounts of nitrogen were increased from 0.0 to 230 p.p.m., but higher increases to 855 p.p.m. showed slight weight reductions. BECKENBACH *et al.* (**3, 4, 5**) observed in corn (*Zea mays*) that

plant weights, nitrate concentrations and total nitrogen in the tissues increased with higher amounts of nitrates in the substratum. BARTHOLOMEW *et al.* (**2**) observed that tomato plants grown in nutrient solutions with an abundant supply of nitrogen and potassium produced greater yields of dry matter and had a higher percentage of nitrogen in the leaves than those supplied with low nitrogen.

Absorption of nitrate nitrogen and other anions is associated with proportional energy expenditure, according to LUNDEGARDH (**19**) which is indicated as moles of respired CO_2 per mole of NO_3 or of other anions. His contention is that roots, being negatively charged, absorb metallic cations by attraction; whereas anions are repelled from the negative surface and an extra supply of energy, provided by carbohydrate oxidation in respiration, is needed to overcome the resistance. However, this view of LUNDEGARDH has been challenged by STEWARD (**34**) and HOAGLAND and STEWARD (**15**), who claim that their experiments on barley roots and on disks of potatoes have not led to a fundamental distinction between cation and anion accumulation; moreover, the cation ammonium and the anion nitrate usually produced the most marked acceleration of respiration over that occurring in distilled water. GREGORY (**12**) claims that nitrogen deficiency in barley always leads to a reduction in the rate of respiration. MCCALLA and WOODFORD (**22**) state that when nitrogen was limited more phosphorus and sulphur were taken in by wheat plants than when nitrogen levels were high. ROSE and MCCALLA (**25**) found that limiting nitrogen reduced the size of plants and the amounts of all nutrients absorbed except phosporus. SHANK (**26**) observed that top to root ratios increased with an increase in nitrogen concentration, and he attributes this condition to the failure of translocation to leaves of substances absorbed by roots because of insufficiency for optimal growth.

ARNON (**1**), discussing the merits of nitrate *vs.* ammonium sources in the nutrition of barley and obtaining a more satisfactory growth with the former than latter, claims that "furnishing the plant with nitrogen exclusively in the reduced form of ammonium may be regarded as not merely a substitution of one source of nitrogen for another but a deprivation of the plant of an oxidizing agent." That nitrates might act in the capacity of oxidizing agents is indicated by MEYERHOF'S computations (**21**) of WARBURG and NEGELEIN'S (**36**) results which show that, of the energy released by the oxidation of carbon to CO_2, only 30 per cent. is used in the reduction of NO_3^- to NH_4^+. HAMNER (**13**) found that wheat plants supplied with nitrate assimilated approximately 56.0 per cent. and liberated by their roots 27.0 and tops 66.0 per cent. more carbon dioxide than similar plants grown in minus-nitrate cultures. However, in the absence of similar cultures with ammonium cations, one is unable to attribute the gain in respiration to the nitrate ions alone or to an increased metabolic activity resulting from a greater protoplasmic volume by the addition of nitrogen to the cultures. Recent studies by GILBERT and SHIVE (**11**) have indicated that production

of CO_2 by roots was increased with a greater nitrate uptake but not with ammonium.

The utilization of oxygen released by nitrate reduction to ammonium for respiration by roots, mentioned above, has not been investigated, although in *A. comosus* the various phases of nitrate assimilation taking place mostly in the chlorophyllose regions of the leaves (**30**) suggest that any oxygen released by reduction of nitrates might be utilized by the adjacent chlorophyllose tissues or may be transported downward to the roots in solution with other organic solutes.

Comparison of these results with those of the various investigators, above mentioned, shows that a very satisfactory agreement was observed with respect to nitrogen supplies in the nutrient solution and plant weights, intake of total nitrogen in both nitrate and ammonium series, and potassium and calcium in the nitrate series. Total phosphorus absorption was greater in the high-N than low-N cultures of the nitrate series, which was not in agreement with the results of McCALLA and WOODFORD (**22**) and ROSE and McCALLA (**25**). The differences in pH shifts of nutrient solutions supplied with nitrate or ammonium salts have been discussed with respect to iron solubility and availability to plants. BRIGGS (**7**) observed in *Tropaeolum majus* that boron in small amounts may greatly enhance nitrate intake by roots.

The relations between cation and anion accumulation in plant tissues have been discussed by HOAGLAND and BROYER (**14**) who state that "in a number of experiments on root tissues possessing a high potentiality for salt absorption, K and Br were withdrawn from the solution in nearly equivalent quantities, although various secondary effects may complicate the study of this relation by causing K losses from cells, etc." The same authors, commenting on nitrate absorption and accumulation, state that "the concentration of NO_3 in the sap increased very rapidly during the period of 16 hours then fell off abruptly, presumably the rate of NO_3 reduction in the tissues greatly exceeded the rate of NO_3 accumulation." LUNDEGARDH (**18, 20**), discussing the rôle of cations on the electrostatic charges of cell membranes which influence anion attraction, claims that "bivalent ions, such as Ca^{++} and Mn^{++}, attract water molecules from the surroundings and consequently dehydrate the membrane, causing the molecules to pack closer together and increase the charge of the membrane, which is a function of the number of valences per unit of area." Regarding potassium, he states that "a membrane in which the free valences are chiefly saturated with K^+ has a looser constitution, the electrical charge is lower and the membrane probably does not resist high charges." Further evidence on the nature of the negative charge of plant cells is offered by OSTERHOUT (**23**), who states that "remarkable changes are brought about by dilute solutions of KOH but not as effectively by NaOH in transforming the negative cells of Nitella to positive cells by dissolving out a fatty acid, a constituent of the protoplasm charged negatively." Moreover, the same author (**23**) claims, on the basis of ionic

mobilities, that "the protoplasmic surface cannot be a pore system, for in such a system all cations must have greater mobilities than all anions or vice versa." CONWAY (**8**), attempting to explain the differential rate of ion penetration through membranes as being due to different diameters of the ions, gives the following relative diameters referred to K-ion as unity: K = 1.00; Na = 1.49; Ca = 2.51; and Mg = 2.81. The relative diameters of the ions mentioned by CONWAY are different from the ionic radii of the same elements of PAULING (**24**) with values in Å as follows: K = 1.33; Mg = 0.65; Ca = 0.99; NO_3 = 1.21, etc. The data in table V indicate a ratio of potassium

TABLE V

AMOUNTS OF ELEMENTS PER PLANT AND RATIOS OF ELEMENTS ABSORBED

ELEMENTS GM./PLANT	NITROGEN				POTASSIUM†			
	N-N		A-N		N-N		A-N	
	HIGH-N*	LOW-N*	HIGH-N	LOW-N	HIGH-K	LOW-K	HIGH-K	LOW-K
K	18.79	17.59	13.28	25.52	43.55	5.97	39.44	7.13
Ca	6.68	1.12	0.73	1.09	1.78	4.00	1.18	1.05
Mg	1.04	1.05	0.97	1.42	0.70	1.42	0.88	1.19
N	8.64	1.78	9.76	1.82	5.99	4.68	5.82	5.78
P	0.77	0.42	1.01	0.53	0.53	0.25	0.64	0.31
Ratios								
K/Ca	2.81	15.70	18.20	23.40	24.50	1.50	33.50	6.80
K/N	2.19	9.88	1.36	14.00	7.27	1.28	6.78	1.23
Ca/N	0.77	0.63	0.08	0.60	0.30	0.86	0.20	0.18

* High-N and K = 140.0 mg. N and 200 mg. K per liter, respectively.
Low-N and K = 2.8 gm. N and 4 mg. K per liter, respectively.
† Reference (**33**).

to calcium of 2.81 (18,787 ÷ 6,680), for the high-N cultures in the nitrate series, differing from the theoretical ratio of ionic diameters (2.51) proposed by CONWAY, 12.0 per cent. The ratio of K to Mg for the same culture and series was 17.3 : 1 (18,787 ÷ 1,039) which is 509 per cent. greater than that postulated by the diameter ratios of K^+ to Mg^{++} ions. Similar ratios for elements absorbed from the low-N cultures in the nitrate series or in the ammonium series were not in agreement with the diameter ratios of the ions, possibly because the amounts of NO_3^- ions in the low-N cultures were abnormally low with respect to the various cations, or the antagonistic effects of NH_4^+ ions in the ammonium series interfered with cation absorption. Further evidence of ion interrelationships, indicated by the total amounts of certain nutrient elements in the tissues of various cultures, is presented in table IV, which shows that ratio values of potassium to calcium were not in agreement with the theoretical relative diameter 2.51 except for the high-N culture in the N-n series. Potassium to nitrogen ratios also deviated appreciably from the equimolecular value of 2.785 (39 ÷ 14), being high in cultures with too little nitrogen or too much potassium and low in those with too much nitrogen or too little potassium. Calcium to nitrogen ratios show that the high ammonium content of the high-N culture in the A-n series, and also a similar

content of potassium ions of the high-K culture in the same series depressed greatly calcium absorption. Phosphorus values in the tissues were almost twice as great for the high-N or high-K than for the low-N or low-K cultures, indicating that high concentrations of NH_4 ion enhanced intake of PO_4 ions and also that similar concentrations of NO_3 ions had not interfered with the absorption of PO_4 ions from solution culture.

Comparison of the data under nitrogen and potassium (**33**) in table V shows that the chemical composition of plant tissues in mineral nutrient elements is not constant but varies widely and depends on the amounts of such elements taken in by the roots. High concentrations of NH_4 or K cations reduce the intake of Ca cations and enhance that of PO_4 anions.

Summary

1. *A. comosus* grown in solution cultures supplied with 140.0 or 2.8 mg. of nitrogen per liter either as nitrate or ammonium produced, after one year's growth, greater weights in the high- than low-nitrogen cultures. Nitrogen absorption from nutrient solutions was approximately five times greater for the high-nitrogen (140.0 mg.) than low-nitrogen (2.8 mg.) cultures in both series.

2. Total ash content per plant was higher in the high-nitrogen cultures of the nitrate series and in the low-nitrogen cultures of the ammonium series than in the competing cultures, possibly because of NO_3 anions attracting cations and NH_4 cations repelling similar cations in the nitrate and ammonium series, respectively.

3. Potassium values per plant were approximately the same for the high-nitrogen and low-nitrogen cultures in the nitrate series, but in the ammonium series they were approximately 92.0 per cent. greater for the low-nitrogen cultures; presumably high NH_4 ion concentrations caused inhibition of K-ion intake.

4. Calcium values per plant were greater for the high- than low-nitrogen cultures in the nitrate series. In the ammonium series calcium values for the high-nitrogen cultures were approximately two-thirds as great as in the low-nitrogen cultures, resulting, presumably, from the antagonistic effects of high concentrations of NH_4 ions in the high nitrogen cultures.

5. Magnesium absorption per plant from nutrient solutions was approximately the same for the high- and low-nitrogen cultures in the nitrate series, but in the ammonium series it was 1.475 times as great for the low- as for the high-nitrogen cultures.

6. Phosphorus content per plant was 1.81 and 1.72 times greater for the high- than low-nitrogen cultures in the nitrate and ammonium series, respectively.

7. Iron content per plant was greater for the cultures in the nitrate than ammonium series, but in the former series 90.3 per cent. of it was in the roots whereas, in the latter series, the roots of the high-nitrogen cultures contained 38.5 per cent. of total plant iron and those of the low-nitrogen cultures

89.5 per cent. Translocated iron in the leaves as percentage of total iron was 8.6 or 8.8 in the nitrate series and 57.5 or 10.0 per cent. in the ammonium series for the high-nitrogen or low-nitrogen cultures, respectively.

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